Diffusion tensor MRI tractography reveals increased fractional anisotropy in arcuate fasciculus following music-cued motor training

Citation for published version:
Moore, E, Schaefer, R, Bastin, M, Roberts, N & Overy, K 2017, 'Diffusion tensor MRI tractography reveals increased fractional anisotropy in arcuate fasciculus following music-cued motor training', *Brain and Cognition*, vol. 116, pp. 40-46. https://doi.org/10.1016/j.bandc.2017.05.001

Digital Object Identifier (DOI):
10.1016/j.bandc.2017.05.001

Brain and Cognition 116 (2017) 40–46

Contents lists available at ScienceDirect

Brain and Cognition

journal homepage: www.elsevier.com/locate/b&c

Diffusion tensor MRI tractography reveals increased fractional anisotropy (FA) in arcuate fasciculus following music-cued motor training

Emma Moore[a], Rebecca S. Schaefer[b,c], Mark E. Bastin[d], Neil Roberts[e], Katie Overy[a,f,*]

[a] Institute for Music in Human and Social Development (IMHSD), Reid School of Music, University of Edinburgh, Edinburgh, UK
[b] Health, Medical and Neuropsychology Unit, Institute of Psychology, Faculty of Social and Behavioural Sciences, Leiden University, Leiden, The Netherlands
[c] Leiden Institute for Brain and Cognition (LIBC), Leiden University, Leiden, The Netherlands
[d] Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK
[e] Clinical Research Imaging Centre (CRIC), University of Edinburgh, Edinburgh, UK
[f] Department of Music Education, Don Wright Faculty of Music, University of Western Ontario, London, Canada

ARTICLE INFO

Keywords:
Neuroplasticity
Arcuate fasciculus
Music-cued motor training
White matter
Diffusion tensor MRI
Auditory-motor training

ABSTRACT

Auditory cues are frequently used to support movement learning and rehabilitation, but the neural basis of this behavioural effect is not yet clear. We investigated the microstructural neuroplasticity effects of adding musical cues to a motor learning task. We hypothesised that music-cued, left-handed motor training would increase fractional anisotropy (FA) in the contralateral arcuate fasciculus, a fibre tract connecting auditory, pre-motor and motor regions. Thirty right-handed participants were assigned to a motor learning condition either with (Music Group) or without (Control Group) musical cues. Participants completed 20 minutes of training three times per week over four weeks. Diffusion tensor MRI and probabilistic neighbourhood tractography identified FA, axial (AD) and radial (RD) diffusivity before and after training. Results revealed that FA increased significantly in the right arcuate fasciculus of the Music group only, as hypothesised, with trends for AD to increase and RD to decrease, a pattern of results consistent with activity-dependent increases in myelination. No significant changes were found in the left ipsilateral arcuate fasciculus of either group. This is the first evidence that adding musical cues to movement learning can induce rapid microstructural change in white matter pathways in adults, with potential implications for therapeutic clinical practice.

1. Introduction

Moving physically to a steady beat is a universal human phenomenon, often occurring spontaneously and enjoyably in a musical context (Chen, Zatorre, & Penhune, 2006; Schaefer & Overy, 2015). Accordingly, auditory cues are increasingly used to support movement learning and rehabilitation (Schaefer, 2014), with evidence suggesting that musical stimuli can support physical exercise (Karageorghis & Priest, 2012), movement rehabilitation after stroke (Thaut, 2005) and improve gait in patients with Parkinson's disease (Benoit et al., 2014; Thaut et al., 1996; Dalla Bella, Benoit, Farrugia, Schwartz, & Kotz, 2015). However, the neural basis of effective auditory-cued motor training is not yet fully understood (Schaefer, Morcom, Roberts, & Overy, 2014).

A range of evidence suggests that high levels of musical training are associated with neural differences in motor circuitry, including corticospinal tracts (Imfeld, Oechslin, Meyer, Loenneker, & Jancke, 2009), pyramidal tracts (Rüber, Lindenberg, & Schlaug, 2013), corpus callosum (Schlaug, Jäncke, Huang, Staiger, & Steinmetz, 1995; Schmithorst & Wilke 2002; Steele, Bailey, Zatorre, & Penhune, 2013), and internal capsule (Bengtsson et al., 2005; Han et al., 2009) (for a review see Moore, Schaefer, Bastin, Roberts, & Overy, 2014). Further evidence suggests that musical training can specifically affect auditory-motor circuitry (Bangert et al., 2006; Baumann et al., 2007; Chen, Penhune, & Zatorre, 2008; Herholz, Coffey, Pantev, & Zatorre, 2016; Palomar-Garcia, Zatorre, Ventura-Campos, Bueicheku, & Avila, 2016; Zatorre, Chen, & Penhune, 2007). For example, short-term piano training has been shown to lead to co-activation of auditory and motor regions during music listening tasks (Bangert & Altenmüller, 2003; Lahav, Saltzman, & Schlaug, 2007) and enhanced activation in premotor cortex and brain areas associated with sensorimotor integration (Herholz et al., 2016). In individual patient case studies, Melodic Intonation Therapy (MIT) (Albert, Sparks, & Helm, 1973), a speech therapy method involving synchronised singing and tapping, has been found to lead to an increased number of fibres and increased tract volume of the arcuate fasciculus, a major fibre tract connecting auditory

* Corresponding author at: Institute for Music in Human and Social Development, Reid School of Music, University of Edinburgh, Alison House, 12 Nicolson Square, Edinburgh EH8 9DF, UK.
E-mail address: K.Overy@ed.ac.uk (K. Overy).

http://dx.doi.org/10.1016/j.bandc.2017.05.001
Received 16 January 2017; Received in revised form 5 May 2017; Accepted 5 May 2017
0278-2626/

and motor brain regions (Schlaug, Marchina, & Norton, 2009; Zipse, Norton, Marchina, & Schlaug, 2012). Highly trained musicians have also been found to show higher fractional anisotropy (FA) values and increased tract volume in the arcuate fasciculus, when compared with non-musicians (Halwani, Loui, Rüber, & Schlaug, 2011). Recent research confirms a posterior termination of the arcuate fasciculus in the superior temporal gyrus, containing primary auditory cortex (Maffei, Soria, Prats-Galino, & Catani, 2015), and anterior termination in the inferior precentral gyrus, containing both primary and premotor regions (Brown et al., 2014). The arcuate fasciculus thus presents a strong candidate tract for the potential neuroplasticity effects of auditory-cued motor training. Despite this, to our knowledge, no controlled, longitudinal studies have yet investigated this possibility (Moore et al., 2014).

The aim of the present study was to use diffusion tensor MRI (DT-MRI) and probabilistic neighbourhood tractography (PNT; Clayden et al., 2011) to investigate whether a short period of left-handed, music-cued motor training would induce increased FA in the contralateral but not ipsilateral arcuate fasciculus. DT-MRI and tractography allow for detailed exploration of the white matter structure of the brain via measurement of the direction and magnitude of water molecule diffusion in segmented tracts-of-interest (Clayden, Storkey, Maniega, & Bastin, 2009). In white matter, water molecule motion is restricted such that diffusion is greater along than perpendicular to the principal fibre direction. FA measures the directionality coherence of water molecule diffusion and is frequently used to infer information about white matter structure and connectivity (Basser, 1995), while mean (MD), axial (AD) and radial (RD) diffusivity measure the total magnitude of water diffusion and its components parallel and perpendicular to the principal fibre direction, respectively (Song et al., 2002). Together these parameters can provide an indication of levels of myelination, axonal membrane integrity and other underlying biological structures (Beaulieu, 2002; Beaulieu, 2014; Song et al., 2002; Wheeler-Kingshott & Cercignani, 2009). PNT has several advantages over region-of-interest, voxel-based and deterministic tractography methods, including automatic tract segmentation rather than manual seed-point placement (thus reducing observer bias) and tract segmentation in native space rather than standard space, thereby allowing subtler changes in white matter microstructure to be detected (Clayden et al., 2011).

We designed a novel training paradigm in which participants learned four sequences of eight finger-to-thumb opposition movements with their left, non-dominant hand, using a visual display either with (Music group) or without (Control group) musical cues. To explore changes in the microstructural properties of the arcuate fasciculus we compared FA, AD and RD biomarkers, obtained using PNT, in bilateral arcuate fasciculi of both groups before and after training. We predicted that left-handed, music-cued motor training would lead to increased FA specifically in the right arcuate fasciculus of the Music group only.

2. Materials and methods

2.1. Participants

Thirty healthy volunteers aged 18–30 years were recruited using an online student recruitment website at the University of Edinburgh, UK. All participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971; mean = 81.90, SD = 17.26) and had no history of neurological or psychiatric disorders. None of the participants had more than six years (mean = 1.41, SD = 1.56 years) of musical training and none were currently practising or learning to play a musical instrument. The participants were quasi-randomly assigned (according to the order of recruitment) to either the Music (n = 15; mean age = 21.27, SD = 1.98 years; 4 male) or Control (n = 15; mean age = 21.33, SD = 2.38 years; 5 male) groups. All participants gave informed consent of their willingness to participate and were reimbursed for their practice time and travel expenses for attending the two MRI scans. The study was carried out in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the University of Edinburgh and the West of Scotland Research Ethics Committee, UK (REC reference number 12/WS/0229).

Table 1
The four motor sequences of eight finger-to-thumb opposition movements.

Sequence 1	1, 3, 2, 4, 3, 1, 3, 2
Sequence 2	2, 4, 3, 4, 1, 1, 2, 3
Sequence 3	3, 4, 1, 2, 4, 4, 2, 1
Sequence 4	4, 2, 1, 3, 2, 3, 1, 4

2.2. Stimuli

The training paradigm involved learning four sequences of eight finger-to-thumb opposition movements (shown in Table 1) with the left (non-dominant) hand (in order to allow greater potential for improvement). For the purposes of the study, the second to fifth digits (i.e. index to pinkie fingers) of the left hand were labelled from 1 to 4, respectively. All participants were asked to practice the four sequences with their left hand for 20 min, three times per week over a four-week period.

For each sequence, an animated visual display was created consisting of four vertical lines, one to represent each finger. Circles descended the vertical lines onto a horizontal line near the bottom of the screen, at which point participants touched the appropriate finger to their thumb (Fig. 1), synchronising with the visual display. For the Music group there was an additional soundtrack providing temporal cues for each finger movement and pitch cues to indicate the correct finger to move, thus establishing an auditory-motor relationship between the musical cues and corresponding finger movements. To ensure that the four digits were equally involved during training, each individual finger was used at the start of one sequence, at the end of another sequence and appeared a total of eight times overall within the four sequences.

2.3. Procedure

Following recruitment and confirmation of inclusion criteria, participants underwent an initial MRI scanning session and behavioural assessment. Training then consisted of watching the videos online and practising the appropriate motor sequences, followed by logging progress. Each video sequence was identified by the starting finger and had

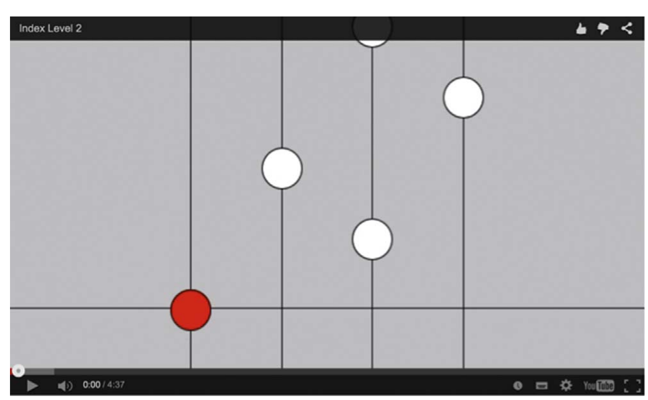

Fig. 1. A snap-shot 'still' from one of the training videos. From left to right the four vertical lines represent the index to pinkie fingers of the left hand. As the video plays, the circles move down the screen and when they reach the horizontal line the participant moves the appropriate finger to touch the thumb; in the auditory-motor condition this corresponds with an appropriate pitch cue and a steady beat. The red circle denotes the start of the sequence.

four levels of increasing difficulty, implemented by increasing the speed of the visual display and tempo of the music (to ensure that the motor exercise remained challenging throughout the four-week training period). Each video ended with a so-called "30-s challenge", also used as a behavioural measure (see Section 2.4). Progression to each difficulty level was determined via self-assessment, whereby participants moved up a level (i.e. moved to a higher speed) once they were comfortably able to complete the preceding level. None of the participants reached the final, most difficult level of training, indicating that the task was sufficiently challenging and fit for purpose. Participants scheduled three practice sessions evenly across the week (for example, on Monday, Wednesday and Saturday) and practice was monitored, with email reminders if practice sessions were missed (two participants, one in each group, only completed 11 out of 12 practice sessions). After two weeks, participants completed a mid-training behavioural test and after four weeks, participants returned for a final behavioural test and a second MRI scanning session.

2.4. Behavioural assessment and analysis

Following Karni et al. (1995), the measure chosen to assess motor performance was the accurate completion of as many repetitions of a particular sequence as possible during a 30-s time period, referred to as the "30-s challenge" and identical to the practice task at the end of each training video. Each sequence was displayed as a static visual image, for all participants. Performance was assessed pre-, mid- and post-training and included four Untrained sequences as well as the four Trained sequences (in order to test how well participants could apply their new motor skill to an unfamiliar finger movement sequence). Thus, each behavioural assessment consisted of eight 30-s challenges, with the sequences presented in a different, counter-balanced order for each participant. Data were recorded using an adapted Peregrine Gaming Glove© (http://theperegrine.com) with software specifically designed to register finger-to-thumb touches, developed by Minerva Design, UK. For verification purposes, video data of the behavioural assessments were obtained using a Canon PowerShot SX240 HS digital camera mounted on a tripod.

The number of correctly performed sequences for each trial was extracted and averaged for the Trained and Untrained sequences pre-, mid- and post-training using MATLAB 2010a (Natick, USA). The number of correct sequences performed for each sequence type (Trained and Untrained) was compared between time-points (pre-, mid- and post-training) and groups (Music and Control) using $3 \times 2 \times 2$ mixed ANOVA. Shapiro-Wilk Tests were used to confirm that the data were normally distributed, thus establishing the suitability of parametric statistics, while Mauchly's Tests of Sphericity were used to check homogeneity of variance for ANOVA. Statistical analysis was performed using IBM SPSS Statistics for Macintosh (Version 21.0, IBM Corp., 2012; http://www.ibm.com/software/uk/analytics/spss) with a significance threshold of $p < 0.05$.

2.5. Magnetic resonance imaging acquisition

DT-MRI data were acquired pre- and post-training at the Clinical Research Imaging Centre (CRIC), University of Edinburgh, UK (http://www.cric.ed.ac.uk), using a Siemens Magnetom Verio 3T MRI scanner (http://www.healthcare.siemens.co.uk) with 12-channel matrix head coil. The whole brain DT-MRI examination consisted of 6 T_2-weighted ($b = 0\,s\,mm^{-2}$) and sets of diffusion-weighted ($b = 1000\,s\,mm^{-2}$) single-shot spin-echo echo-planar imaging (EPI) volumes acquired with diffusion gradients applied in 56 non-collinear directions. Volumes were acquired in the axial plane, with a field-of-view of 240×240 mm, 55 contiguous slice locations, and image matrix and slice thickness designed to give 2.5 mm isotropic voxels. The repetition time (TR) and echo time (TE) for each EPI volume were 10.2 s and 69.6 ms respectively, with a total acquisition time of 11.03 min. The bandwidth was 2126 Hz/Pixel.

2.6. Quantitative tractography analysis

All DT-MRI data were converted from DICOM (http://dicom.nema.org) to NIfTI-1 (http://nifti.nimh.nih.gov/nifti-1) format using the TractoR package for fibre tracking analysis (http://www.tractor-mri.org.uk). FSL tools (http://www.fmrib.ox.ac.uk/fsl) were then used to extract the brain, remove bulk subject motion and eddy current induced distortions by registering all subsequent volumes to the first T_2-weighted EPI volume (Jenkinson & Smith, 2001), estimate the water diffusion tensor and calculate parametric maps of FA, AD and RD from its eigenvalues using DTIFIT.

Tract-average measures of the three water diffusion biomarkers within the bilateral arcuate fasciculi were determined using probabilistic neighbourhood tractography (PNT; Clayden et al., 2011). The principal advantage of PNT compared with region-of-interest and voxel-based methods (e.g. Tract-Based Spatial Statistics; Smith et al., 2006) is the automatic tract segmentation method, which can identify the same fasciculus in different participants using probabilistic tract shape modelling. This reduces observer bias in comparison with methods that, for example, require manual seed-point placement to initiate tracking within fasciculi-of-interest. In addition, PNT segments the tract of interest in native space, rather than standard space, thereby allowing subtle changes to be detected as well as accounting for individual differences in white matter structure and potentially providing a more accurate representation of underlying anatomy, compared with standard space techniques. Re-test coefficients of variance for FA values obtained using PNT have previously been reported to be comparable with other analysis methods (Clayden et al., 2009).

In PNT, multiple native space seed points are automatically placed in a neighbourhood surrounding a seed point transferred from Montréal Neurological Institute standard space for each tract-of-interest. The reconstructed tracts from each seed-point are then compared and the tract that best matches a predefined reference tract in terms of both length and shape selected automatically from this group of 'candidate' tracts. Using the TractoR package (http://www.tractor-mri.org.uk), models of the bilateral arcuate fasciculi were segmented for each subject from underlying connectivity data produced using FSL's BedpostX/ProbTrackX algorithm with a two fibre model per voxel (Behrens et al., 2003) by generating a set of candidate tracts from a $7 \times 7 \times 7$ neighbourhood of voxels centred on the reference seed point (right (38, −44, 24 mm) and left (−36, −40, 28 mm) arcuate fasciculus). The seed point that produced the best-matched tract to the reference was then passed back to FSL's BedpostX/ProbTrackX algorithm to generate a tract mask from which tract-averaged values of FA, AD and RD, weighted by connection probability, were determined for the left and right arcuate fasciculi. All segmented tracts were visually assessed by an experienced operator (MEB) who was blinded to each subject's group status. Tracts that were not an anatomically plausible representation of the fasciculus-of-interest were removed from further analysis, an approach that removes any noise in the data caused by the inclusion of water diffusion biomarker measurements from structures not within the tract of interest. An example of the right arcuate fasciculus segmented using PNT in a representative subject is shown in Fig. 2.

2.7. Statistical analysis of imaging data

Statistical analysis was performed using IBM SPSS Statistics for Macintosh (Version 21.0, IBM Corp., 2012). Shapiro-Wilk tests were used to confirm that all data were normally distributed, whilst Mauchly's Tests of Sphericity or Levene's Test of Variance Equality, as appropriate, tested homogeneity of variance. Comparisons of FA, AD and RD values from the bilateral arcuate fasciculi were made between groups (Music and Control) and time-points (pre- and post-training) separately for each hemisphere using 2 (time) × 2 (group) mixed ANOVAs with a significance threshold of $p < 0.05$.

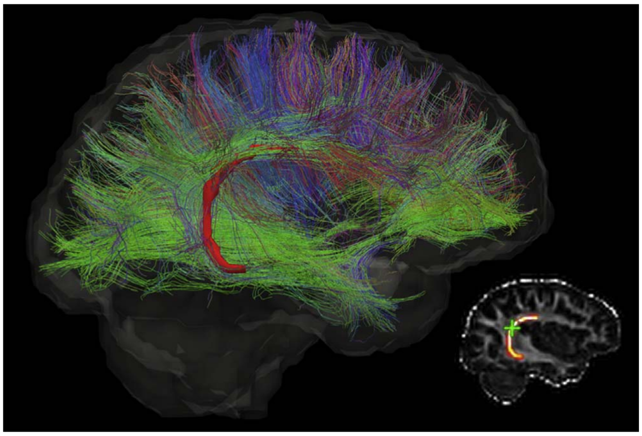

Fig. 2. Whole brain white matter map with right arcuate fasciculus identified from probabilistic neighbourhood tractography (insert) indicated in red for a 23 year-old female volunteer.

3. Results

3.1. Participants

Following appropriate screening of the reconstructed bilateral arcuate fasciculi by an experienced observer (see Section 2.6), three participants were excluded from further analysis, resulting in 14 participants in the Music group (mean age = 21.36, SD = 2.02 years; 3 male) and 13 in the Non-Music group (mean age = 21.54, SD = 2.47 years; 4 male). Independent samples *t*-tests (two-tailed) revealed no significant between-group differences in age, musical experience, initial motor performance or any of the DT-MRI biomarkers in bilateral arcuate fasciculi prior to training.

3.2. Behavioural results

At the end of the training period participants reached varying levels of difficulty on the training videos (levels one, two and three, but not four). The mean number of motor sequences performed correctly in 30 s during the behavioural test was calculated for each group at each time point for both Trained and Untrained sequences (Fig. 3). Mauchly's test indicated that assumption of sphericity had been violated thus Greenhouse-Geisser corrections were applied. A 3 × 2 × 2 ANOVA revealed a main effect of time-point ($F(1.52,37.90) = 393.90$; $p < 0.001$) and sequence type (Trained or Untrained) ($F(1,25) = 100.53$; $p < 0.001$), as well as a significant time-point × sequence type interaction ($F(1.57,39.15) = 92.71$; $p < 0.001$). These results indicate that both groups significantly improved their motor performance for all sequences over time and made significantly greater improvements in Trained sequences compared with Untrained sequences. There were no main effects of group, with no between-group differences at any time-point. Post-hoc 2(sequence type) × 2(group) ANOVAs confirmed a significant effect of sequence type mid ($F(1,25) = 80.36$; $p < 0.001$) and post-training ($F(1,25) = 209.33$; $p < 0.001$) but not pre-training, as would be expected. Pearson's correlation analyses (two-tailed) confirmed a significant relationship between pre and post-training performance across all participants for both Trained ($r(25) = 0.78$; $p < 0.001$) and Untrained sequences ($r(25) = 0.72$; $p < 0.001$), and a significant relationship between pre-training performance and FA in the contralateral, right ($r(25) = 0.54$; $p < 0.01$), but not ipsilateral, left ($p = 0.26$) arcuate fasciculus, indicating the appropriateness of the training paradigm to the tract of interest.

3.3. FA changes in the arcuate fasciculi

Mean tract-averaged FA values in the arcuate fasciculi were calculated for each group pre- and post-training (Table 2). For the right arcuate fasciculus, a mixed 2 × 2 ANOVA revealed a significant effect of group ($F(1,25) = 4.53$; $p = 0.04$) and a significant interaction between group and time-point ($F(1,25) = 4.92$; $p = 0.04$). Post-hoc paired samples *t*-tests (two-tailed) indicated that FA values increased significantly over time in the Music group ($t(13) = 2.36$; $p = 0.04$) with a medium effect size (Cohen's $d = 0.63$) with no significant change in the Control group ($t(12) = -0.97$; $p = 0.35$), supporting the experimental hypothesis (Fig. 4). In order to identify whether changes in FA were related to changes in behavioural performance, Pearson's correlations were conducted for both Trained and Untrained sequences, within each group and across all participants. No significant relationships were found, indicating that the FA changes were specifically associated with the type of training involved (music-cued), rather than with changes in behavioural performance. The equivalent ANOVAs, *t*-tests and correlations were conducted for the FA values from the left arcuate fasciculus, with no significant effects or interactions found for this tract, thus supporting the specificity of the effects of the left-handed music-cued motor training on the right (i.e. contralateral) arcuate fasciculus.

3.4. AD and RD changes in the arcuate fasciculi

In order to investigate the possible microstructural change underlying the observed FA increase, mean tract-averaged AD and RD values in bilateral arcuate fasciculi were calculated for each group pre- and post-training (Table 2). For the right arcuate fasciculus, mixed 2 × 2 ANOVAs revealed a significant interaction between group and time-point for RD ($F(1,25) = 5.46$; $p = 0.03$), whilst for AD there was a trend towards a main effect of group ($F(1,25) = 3.63$; $p = 0.07$). Paired *t*-tests revealed that for the Music group only there was a trend for RD to decrease ($t(13) = -2.07$; $p = 0.06$), with a medium effect size ($d = 0.55$) and AD to increase over the training period ($t(13) = 1.89$; $p = 0.08$), again with a medium effect size ($d = 0.51$), indicating increased water diffusion parallel to the fibre direction. The equivalent ANOVAs and *t*-tests were conducted for the AD and RD values from the left arcuate fasciculus, with no significant effects or interactions found for this tract in either group, indicating that the effects were specific to the right hemisphere.

4. Discussion

To our knowledge this is the first evidence that a short, four-week period of music-cued motor training can induce rapid, localised neuroplasticity in white matter structure. Our initial hypothesis was supported, namely increased FA was found in the right arcuate fasciculus in the Music group but not the Control group, in the absence of any change in left arcuate fasciculus FA (or behavioural differences between groups). The auditory-motor neuroplasticity effects were thus specific to the type of training and to the hemisphere contralateral to the trained hand, and not related to behavioural performance. We interpret this result to reflect increased structural connectivity in the white matter tract connecting auditory and motor regions, after a period of auditory-motor training activity.

The rapid plasticity observed in the arcuate fasciculus lends support to previous research suggesting that white matter differences observed between musicians and non-musicians (Halwani et al., 2011) are likely to be at least partly training-induced, rather than a result of pre-existing differences. Intervention studies of MIT with stroke patients have also reported structural changes in the arcuate fasciculus in individual and small groups of subjects (Schlaug et al., 2009; Zipse et al., 2012), although in these cases the training duration and intensity reported was between 112 and 120 hours over 15–16 weeks, compared with just four hours over a four-week training period employed here. Thus, the present study with 27 healthy adults provides evidence that even a short, relatively low-intensity period of auditory-motor training can induce rapid, training-style related structural changes in the arcuate fasciculus.

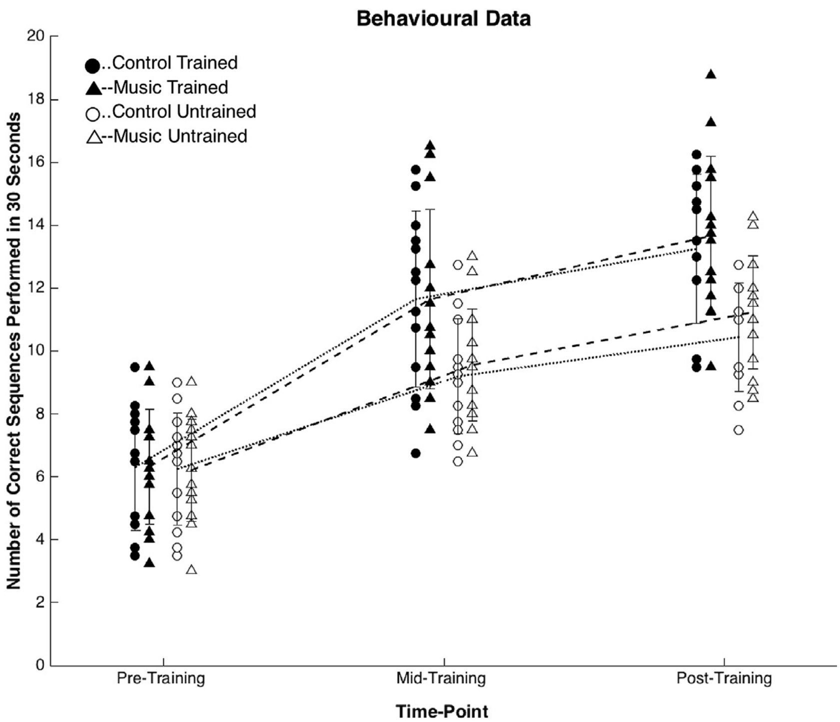

Fig. 3. Scatter and line graph showing the number of correct Trained and Untrained sequences performed within 30 s by the Music (n = 14) and Control (n = 13) groups pre-, mid- and post-training. Error bars represent ± 1 SD.

Table 2
Mean FA, AD and RD values (SD) in bilateral arcuate fasciculi for both groups pre- and post-training, and p values showing the significance of any change between time-points.

		Right arcuate fasciculus				Left arcuate fasciculus			
	Group	Pre	Post	t value	p value	Pre	Post	t value	p value
FA	Music	0.47 (0.03)	0.49 (0.04)	2.36	**0.04**	0.49 (0.04)	0.48 (0.04)	−0.55	0.60
	Control	0.46 (0.03)	0.45 (0.04)	−0.97	0.35	0.49 (0.04)	0.49 (0.03)	−0.49	0.63
AD $10^{-6}\,mm^2\,s^{-1}$	Music	1126.00 (38.85)	1142.00 (28.35)	1.89	**0.08**	1170.63 (50.36)	1170.53 (39.82)	−0.01	0.99
	Control	1106.76 (52.21)	1106.20 (43.83)	−0.05	0.96	1166.50 (49.64)	1147.81 (50.90)	−1.38	0.19
RD $10^{-6}\,mm^2\,s^{-1}$	Music	524.93 (33.67)	507.69 (39.53)	−2.07	**0.06**	519.61 (35.18)	527.98 (38.82)	0.93	0.37
	Control	518.75 (23.23)	529.60 (36.51)	1.25	0.24	517.69 (29.47)	517.30 (26.02)	−0.07	0.94

This four-week timeframe is consistent with Scholz, Klein, Behrens, and Johansen-Berg (2009), who reported increases in FA in white matter underlying the right posterior intraparietal sulcus following six weeks of juggling training, and Langer, Hänggi, Müller, Simmen, and Jäncke (2012), who reported decreases in left (contralateral) corticospinal tract FA following 16 days of right upper-limb immobilisation.

Elucidation of the potential mechanism causing an increase in FA is complex and will require further work. Several mechanisms could be involved (Jones, Knösche, & Turner, 2013; Wheeler-Kingshott & Cercignani, 2009) including increased myelination, changes in axonal membrane structure, increases in axon diameter, fibre packing density or increased fibre number (Beaulieu, 2002; Beaulieu, 2014; Jones et al., 2013; Wheeler-Kingshott & Cercignani, 2009). The growth of new axons in the mature brain is unlikely for this study, given the short four-week training period (Wang, Casadio, Weber, Mussa-Ivaldi, & Parrish, 2014), thus increased fibre numbers is unlikely to have caused the observed changes. Increased myelination, axon diameter or fibre packing density, or changes in the axonal membrane structure could all potentially underlie our FA result.

Importantly though, in addition to significantly increased FA in the right arcuate fasciculus, we found increased AD and decreased RD at trend level. This pattern is indicative of increased water molecule diffusivity parallel to the main fibre direction and suggests the possibility of increased myelination (Song et al., 2002). Myelination is associated with impulse conduction velocity (Ullén, 2009) and is crucial for both the rapid transfer (Lundgaard et al., 2013) and synchronisation (Fields, 2008) of information between different brain regions. Myelination has been shown to be essential for learning new motor tasks in rats (McKenzie et al., 2014), while learning a unilateral motor reaching task has previously been shown to result in increased FA and myelination in the rat brain, specifically in white matter underlying sensorimotor areas contralateral to the trained paw (Sampaio-Baptista et al., 2013). These results are consistent with our finding of a significant increase in FA contralateral to the trained hand, confirming that the changes in water diffusion biomarkers observed in the present study may reflect learning-related increases in myelination. Myelination can be modulated by neuronal activity (Chang, Redmond, & Chan, 2016; Lundgaard et al., 2013), and given that the arcuate fasciculus connects auditory and motor brain regions (Brown et al., 2014; Catani & Jones, 2005; Maffei et al., 2015), our music-cued motor task is likely to have increased neural activity along this tract. Thus, we believe that *activity-dependent*

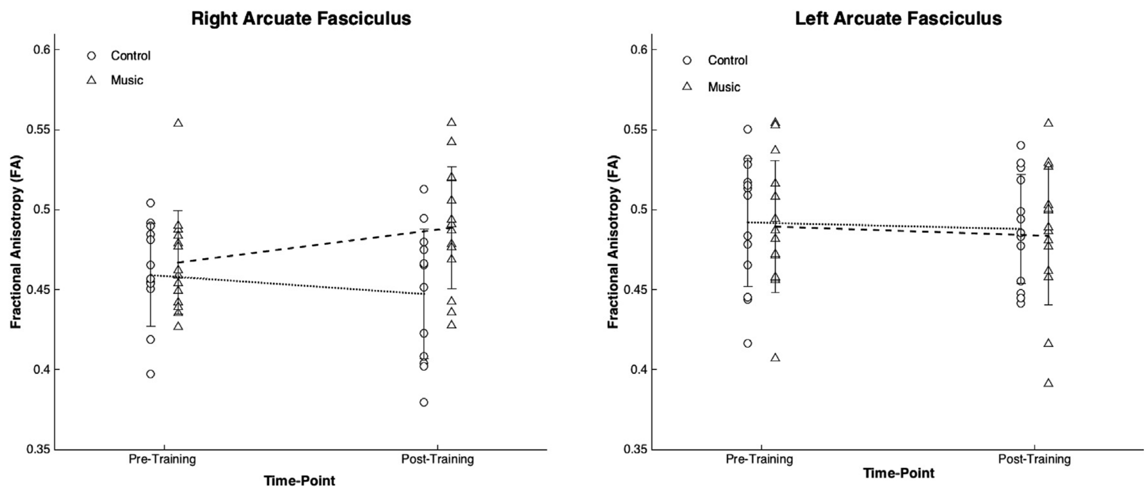

Fig. 4. Scatter and line graphs showing individual and mean FA for participants in the Music (n = 14) and Control (n = 13) groups pre- and post-training in right and left arcuate fasciculus. Error bars represent ± 1 SD.

myelination is the most plausible (though not necessarily the only) mechanism underlying the observed increases in FA and AD and decline in RD.

A further key finding of the present study is that the neuroplasticity effects found in the Music group occurred in the absence of behavioural performance differences between groups or indeed correlations with increases in behavioural performance. This suggests that in the current study, changes observed in FA, AD and RD were related specifically to the style of training (music-cued) rather than the skill level achieved, leading us to conclude that the use of musical cues promoted a style of motor learning involving different perception-action mechanisms, as opposed to promoting better or faster learning. These results may be especially relevant in a rehabilitation context, for example if the desired outcome is improved fronto-temporal connectivity. Future investigation, ideally with larger numbers of participants, of the effects of different kinds of movement cues and longer training periods, may further clarify the specific effects of using auditory cues during motor learning. Importantly, extension of these findings to individuals who need movement rehabilitation may also reveal differences that are specific to particular patient groups.

In a clinical context, these findings may thus have significant implications for post-stroke movement rehabilitation where structural reorganisation is key for functional motor recovery (Dimyan & Cohen, 2011). Persistent, residual impairments in motor function are common in stroke survivors (Broeks, Lankhorst, Rumping, & Prevo, 1999; Wade, Langton-Hewer, Wood, Skilbeck, & Ismail, 1983), thus designing effective rehabilitation interventions to improve recovery prospects is vital. The internet-based training paradigm used in the present study has the potential to be adapted to allow stroke patients to undergo rehabilitation in their own home, whilst receiving support from a therapist. No additional specialist equipment is required, only a computer or tablet and headphones, meaning it has the potential to be cost-effective. We also note that the training paradigm used here was much shorter and less intensive than in studies that have reported changes in arcuate fasciculus following MIT in stroke patients (Norton, Zipse, Marchina, & Schlaug, 2009; Zipse et al., 2012), thus the present study provides important evidence to suggest that microstructural changes in white matter pathways can occur rapidly, at least in young, healthy volunteers. This finding compliments prior work showing that just two to six weeks of music training with adults can lead to significant changes in neural responses to auditory musical stimuli (Bangert & Altenmüller, 2003; Lahav et al., 2007; Lappe, Trainor, Herholz, & Pantev, 2011).

We note that the increase in FA found in the right arcuate fasciculus of the Music group was relatively small. However, the increase was statistically significant ($p < 0.05$), showed a medium effect size ($d = 0.63$), was only found contralaterally, is in line with findings from both animal models and human movement learning studies (e.g. Sampaio-Baptista et al., 2013; Wang et al., 2014) and is further supported by the increase in AD and decrease in RD found at trend level. The relatively small effect may be related to the very short training period and relatively small sample size.

In conclusion, we investigated white matter plasticity in the arcuate fasciculus in the young, healthy adult brain using a novel auditory-motor training paradigm. To our knowledge, this is the first time musical cues have been applied to fine motor skill learning and combined with DT-MRI and PNT in a longitudinal design. We found increased FA in the right arcuate fasciculus following four weeks of left-handed music-cued motor training, suggesting that such motor training can drive rapid microstructural change in relevant white matter pathways. Despite making comparable behavioural improvements, the same structural changes were not observed in the arcuate fasciculus of participants in the Control group who completed identical motor training without musical cueing, suggesting that the effect was specific to the use of musical cues. In the absence of behavioural differences, the neuroplasticity effect seen in the Music group suggests that the use of auditory cues promotes a learning style involving different sensory systems, as opposed to promoting better or faster learning. Our results may be relevant to, for example, post-stroke movement rehabilitation, where structural reorganisation is likely to be essential for physical rehabilitation (Dimyan & Cohen, 2011). Future research will be required to investigate the application of musical cues in movement rehabilitation and to elucidate the optimum type of auditory stimulus to support movement learning and rehabilitation.

Disclosure statement

The authors declare that they have no actual or potential conflicts of interest with this work.

Author contributions

R.S.S., N.R. and K.O. designed the study, R.S.S. and K.O. created the training paradigm. E.M. collected the data, E.M. and R.S.S. analysed the behavioural data, E.M. and M.E.B. analysed the DT-MRI data. All authors contributed to the writing of the paper.

Acknowledgements

This project was supported by the Marie Curie Research Programme

(No. EC FP7-PEOPLE-2010-IEF No. 276529) and Reid School of Music, Edinburgh College of Art (ECA), University of Edinburgh. The study (CRF E131222) was conducted at the Clinical Research Imaging Centre (CRIC) and Wellcome Trust Clinical Research Facility, University of Edinburgh, Western General Hospital. We would like to thank Dr Zack Moir for composing the music used in the training videos and Emma Aitken and Marlen Roehe for their assistance with the analysis of the behavioural data.

References

Albert, M. L., Sparks, R. W., & Helm, N. A. (1973). Melodic intonation therapy for aphasia. *Archives of Neurology*, *29*(2), 130–131.

Bangert, M., & Altenmüller, E. O. (2003). Mapping perception to action in piano practice: A longitudinal DC-EEG study. *BMC Neuroscience*, *4*(1), 26.

Bangert, M., Peschel, T., Schlaug, G., Rotte, M., Drescher, D., Hinrichs, H., ... Altenmüller, E. (2006). Shared networks for auditory and motor processing in professional pianists: evidence from fMRI conjunction. *Neuroimage*, *30*(3), 917–926.

Basser, P. J. (1995). Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. *NMR in Biomedicine*, *8*(7), 333–344.

Baumann, S., Koeneke, S., Schmidt, C. F., Meyer, M., Lutz, K., & Jancke, L. (2007). A network for audio-motor coordination in skilled pianists and non-musicians. *Brain Research*, *1161*, 65–78.

Beaulieu, C. (2002). The basis of anisotropic water diffusion in the nervous system – A technical review. *NMR in Biomedicine*, *15*, 435–455.

Beaulieu, C. (2014). *The biological basis of diffusion anisotropy. diffusion MRI: From quantitative measurement to in vivo neuroanatomy* (2nd ed., pp. 155–183).

Behrens, T. E. J., Woolrich, M. W., Jenkinson, M., Johansen-Berg, H., Nunes, R. G., Clare, S., ... Smith, S. M. (2003). Characterization and propagation of uncertainty in diffusion-weighted MR imaging. *Magnetic Resonance in Medicine*, *50*(5), 1077–1088.

Bella, S. D., Benoit, C. E., Farrugia, N., Schwartz, M., & Kotz, S. A. (2015). Effects of musically cued gait training in Parkinson's disease: Beyond a motor benefit. *Annals of the New York Academy of Sciences*, *1337*(1), 77–85.

Bengtsson, S. L., Nagy, Z., Skare, S., Forsman, L., Forssberg, H., & Ullén, F. (2005). Extensive piano practicing has regionally specific effects on white matter development. *Nature Neuroscience*, *8*(9), 1148–1150.

Benoit, C. E., Dalla Bella, S., Farrugia, N., Obrig, H., Mainka, S., & Kotz, S. A. (2014). Musically cued gait-training improves both perceptual and motor timing in Parkinson's disease. *Frontiers in Human Neuroscience*, *8*, 1–11.

Broeks, J., Lankhorst, G. J., Rumping, K., & Prevo, A. J. H. (1999). The long-term outcome of arm function after stroke: Results of a follow-up study. *Disability and Rehabilitation*, *21*(8), 357–364.

Brown, E. C., Jeong, J. W., Muzik, O., Rothermel, R., Matsuzaki, N., Juhász, C., ... Asano, E. (2014). Evaluating the arcuate fasciculus with combined diffusion-weighted MRI tractography and electrocorticography. *Human Brain Mapping*, *35*(5), 2333–2347.

Catani, M., & Jones, D. K. (2005). Perisylvian language networks of the human brain. *Annals of Neurology*, *57*(1), 8–16.

Chang, K. J., Redmond, S. A., & Chan, J. R. (2016). Remodeling myelination: Implications for mechanisms of neural plasticity. *Nature Neuroscience*, *19*(2), 190–197.

Chen, J. L., Penhune, V. B., & Zatorre, R. J. (2008). Moving on time: Brain network for auditory-motor synchronization is modulated by rhythm complexity and musical training. *Journal of Cognitive Neuroscience*, *20*(2), 226–239.

Chen, J. L., Zatorre, R. J., & Penhune, V. B. (2006). Interactions between auditory and dorsal premotor cortex during synchronization to musical rhythms. *Neuroimage*, *32*(4), 1771–1781.

Clayden, J. D., Maniega, S. M., Storkey, A. J., King, M. D., Bastin, M. E., & Clark, C. A. (2011). TractoR: Magnetic resonance imaging and tractography with R. *Journal of Statistical Software*, *44*(8), 1–18.

Clayden, J. D., Storkey, A. J., Maniega, S. M., & Bastin, M. E. (2009). Reproducibility of tract segmentation between sessions using an unsupervised modelling-based approach. *Neuroimage*, *45*(2), 377–385.

Dimyan, M. A., & Cohen, L. G. (2011). Neuroplasticity in the context of motor rehabilitation after stroke. *Nature Reviews Neurology*, *7*(2), 76–85.

Fields, R. D. (2008). White matter in learning, cognition and psychiatric disorders. *Trends in Neurosciences*, *31*(7), 361–370.

Halwani, G. F., Loui, P., Rüber, T., & Schlaug, G. (2011). Effects of practice and experience on the arcuate fasciculus: Comparing singers, instrumentalists, and non-musicians. *Frontiers in Psychology*, *2*, 156.

Han, Y., Yang, H., Lv, Y. T., Zhu, C. Z., He, Y., Tang, H. H., ... Dong, Q. (2009). Gray matter density and white matter integrity in pianists' brain: A combined structural and diffusion tensor MRI study. *Neuroscience Letters*, *459*(1), 3–6.

Herholz, S. C., Coffey, E. B. J., Pantev, C., & Zatorre, R. J. (2016). Dissociation of neural networks for predisposition and for training-related plasticity in auditory-motor learning. *Cerebral Cortex*, *26*, 3125–3134.

Imfeld, A., Oechslin, M. S., Meyer, M., Loenneker, T., & Jancke, L. (2009). White matter plasticity in the corticospinal tract of musicians: A diffusion tensor imaging study. *Neuroimage*, *46*(3), 600–607.

Jenkinson, M., & Smith, S. (2001). A global optimisation method for robust affine registration of brain images. *Medical Image Analysis*, *5*(2), 143–156.

Jones, D. K., Knösche, T. R., & Turner, R. (2013). White matter integrity, fiber count, and other fallacies: The do's and don'ts of diffusion MRI. *Neuroimage*, *73*, 239–254.

Karageorghis, C. I., & Priest, D. L. (2012). Music in the exercise domain: A review and synthesis (Part I). *International Review of Sport and Exercise Psychology*, *5*(1), 44–66.

Karni, A., Meyer, G., Jezzard, P., Adams, M. M., Turner, R., & Ungerleider, L. G. (1995). Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature*, *377*, 155–158.

Lahav, A., Saltzman, E., & Schlaug, G. (2007). Action representation of sound: Audiomotor recognition network while listening to newly acquired actions. *Journal of Neuroscience*, *27*(2), 308–314.

Langer, N., Hänggi, J., Müller, N. A., Simmen, H. P., & Jäncke, L. (2012). Effects of limb immobilization on brain plasticity. *Neurology*, *78*(3), 182–188.

Lappe, C., Trainor, L. J., Herholz, S. C., & Pantev, C. (2011). Cortical plasticity induced by short-term multimodal musical rhythm training. *PLoS ONE*, *6*(6), e21493 10.1371.

Lundgaard, I., Luzhynskaya, A., Stockley, J. H., Wang, Z., Evans, K. A., Swire, M., ... Káradóttir, R. T. (2013). Neuregulin and BDNF induce a switch to NMDA receptor-dependent myelination by oligodendrocytes. *PLoS Biology*, *11*(12), e1001743.

Maffei, C., Soria, G., Prats-Galino, A., & Catani, M. (2015). Imaging white-matter pathways of the auditory system with diffusion imaging tractography. *The Human Auditory System: Fundamental Organization and Clinical Disorders*, *129*, 277–288.

McKenzie, I. A., Ohayon, D., Li, H., de Faria, J. P., Emery, B., Tohyama, K., & Richardson, W. D. (2014). Motor skill learning requires active central myelination. *Science*, *346*(6207), 318–322.

Moore, E., Schaefer, R. S., Bastin, M. E., Roberts, N., & Overy, K. (2014). Can musical training influence brain connectivity? Evidence from diffusion tensor MRI. *Brain Sciences*, *4*(2), 405–427.

Norton, A., Zipse, L., Marchina, S., & Schlaug, G. (2009). Melodic intonation therapy. *Annals of the New York Academy of Sciences*, *1169*(1), 431–436.

Oldfield, R. C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia*, *9*(1), 97–113.

Palomar-Garcia, M. A., Zatorre, R. J., Ventura-Campos, N., Bueicheku, E., & Avila, C. (2016). Modulation of functional connectivity in auditory-motor networks in musicians compared with non-musicians. *Cerebral Cortex, bhw120*, 1–11.

Rüber, T., Lindenberg, R., & Schlaug, G. (2013). Differential adaptation of descending motor tracts in musicians. *Cerebral Cortex*, *25*(6), 1490–1498.

Sampaio-Baptista, C., Khrapitchev, A. A., Foxley, S., Schlagheck, T., Scholz, J., Jbabdi, S., ... Johansen-Berg, H. (2013). Motor skill learning induces changes in white matter microstructure and myelination. *Journal of Neuroscience*, *33*(50), 19499–19503.

Schaefer, R. S. (2014). Auditory rhythmic cueing in movement rehabilitation: Findings and possible mechanisms. *Philosophical Transactions of Royal Society B*, *369*(1658), 20130402.

Schaefer, R. S., Morcom, A. M., Roberts, N., & Overy, K. (2014). Moving to music: effects of heard and imagined musical cues on movement-related brain activity. *Frontiers in Human Neuroscience, 8*.

Schaefer, R. S., & Overy, K. (2015). Motor responses to a steady beat. *Annals of the New York Academy of Sciences*, *1337*(1), 40–44.

Schlaug, G., Jäncke, L., Huang, Y., Staiger, J. F., & Steinmetz, H. (1995). Increased corpus callosum size in musicians. *Neuropsychologia*, *33*(8), 1047–1055.

Schlaug, G., Marchina, S., & Norton, A. (2009). Evidence for plasticity in white-matter tracts of patients with chronic Broca's aphasia undergoing intense intonation-based speech therapy. *Annals of the New York Academy of Sciences*, *1169*(1), 385–394.

Schmithorst, V. J., & Wilke, M. (2002). Differences in white matter architecture between musicians and non-musicians: A diffusion tensor imaging study. *Neuroscience Letters*, *321*(1), 57–60.

Scholz, J., Klein, M. C., Behrens, T. E., & Johansen-Berg, H. (2009). Training induces changes in white-matter architecture. *Nature Neuroscience*, *12*(11), 1370–1371.

Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., Mackay, C. E., ... Behrens, T. E. (2006). Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *Neuroimage*, *31*, 1487–1505.

Song, S. K., Sun, S. W., Ramsbottom, M. J., Chang, C., Russell, J., & Cross, A. H. (2002). Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage*, *17*(3), 1429–1436.

Steele, C. J., Bailey, J. A., Zatorre, R. J., & Penhune, V. B. (2013). Early musical training and white-matter plasticity in the corpus callosum: Evidence for a sensitive period. *Journal of Neuroscience*, *33*(3), 1282–1290.

Thaut, M. H. (2005). *Rhythm, music, and the brain: Scientific foundations and clinical applications*. New York & London: Routledge.

Thaut, M. H., McIntosh, G. C., Rice, R. R., Miller, R. A., Rathbun, J., & Brault, J. M. (1996). Rhythmic auditory stimulation in gait training for Parkinson's disease patients. *Movement Disorders*, *11*(2), 193–200.

Ullén, F. (2009). Is activity regulation of late myelination a plastic mechanism in the human nervous system? *Neuron Glia Biology*, *5*(1–2), 29–34.

Wade, D. T., Langton-Hewer, R., Wood, V. A., Skilbeck, C. E., & Ismail, H. M. (1983). The hemiplegic arm after stroke: Measurement and recovery. *Journal of Neurology, Neurosurgery and Psychiatry*, *46*, 521–524.

Wang, X., Casadio, M., Weber, K. A., Mussa-Ivaldi, F. A., & Parrish, T. B. (2014). White matter microstructure changes induced by motor skill learning utilizing a body machine interface. *Neuroimage*, *88*, 32–40.

Wheeler-Kingshott, C. A., & Cercignani, M. (2009). About "axial" and "radial" diffusivities. *Magnetic Resonance in Medicine*, *61*(5), 1255–1260.

Zatorre, R. J., Chen, J. L., & Penhune, V. B. (2007). When the brain plays music: Auditory–motor interactions in music perception and production. *Nature Reviews Neuroscience*, *8*(7), 547–558.

Zipse, L., Norton, A., Marchina, S., & Schlaug, G. (2012). When right is all that is left: Plasticity of right-hemisphere tracts in a young aphasic patient. *Annals of the New York Academy of Sciences*, *1252*(1), 237–245.